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For the attention of International Preliminary Examining Authority

Our Ref: **P33779WO/TF/LJP**
Your Ref:

22 June 2004

Dear Sirs

International (PCT) Patent Application No. PCT/GB2003/005158
In the name of Medical Research Council *et al*

I file herewith a Demand for Chapter II PCT.

Arrangements are being made separately by our formalities department for payment of the associated fees. In the event of non-payment or underpayment please debit the requisite sum from our deposit account number 28050204.

An amended set of claims is enclosed, in which claims 1 and 2 have been amended. Claim 1 has been amended to replace "A crystal structure of the pRb/E2F$_{(409-426)}$ complex," with "A crystal comprising a pRb/E2F$_{(409-426)}$ complex, wherein the crystal structure is". Claim 2 has been amended to refer to "A crystal as claimed in claim 1" in view of the amendment made to claim 1.

These amendments have been made to claims 1 and 2 of the present application in light of the International Searching Authority being of the opinion that the subject matter of previous claims 1 and 2 related to the presentation of information. I submit that the subject matter of amended claims 1 and 2 clearly does not relate to the presentation of information because these claims now define a crystal *per se*.

Please acknowledge safe receipt of this letter with enclosures by returning the enclosed copy of EPO Form 1037 duly stamped.

Yours faithfully

FORD, Timothy James
Agent for the Applicants

Enc. Chapter II Demand
EPO Form 1037
Copy letter to International Bureau
Copy claims filed at International Bureau

Claims

1. A crystal comprising a pRb/E2F$_{(409-426)}$ complex, wherein the crystal structure is characterised by the atomic co-ordinates of Annex 1.

2. A crystal as claimed in claim 1, wherein the interactions between E2F$_{(409-426)}$ and pRb comprise one or more of the following interactions:

E2F$_{(409-426)}$ residue	pRb residue
Leu$_{409}$	Lys$_{548}$
Tyr$_{411}$	Glu$_{551}$
Tyr$_{411}$	Ile$_{532}$
Tyr$_{411}$	Glu$_{554}$
His$_{412}$	Arg$_{656}$
His$_{412}$	Lys$_{653}$
Gly$_{414}$	Glu$_{533}$
Gly$_{414}$	Lys$_{652}$
Leu$_{415}$	Leu$_{649}$
Leu$_{415}$	Glu$_{553}$
Leu$_{415}$	Lys$_{537}$
Glu$_{417}$	Lys$_{537}$
Gly$_{418}$	Arg$_{467}$
Glu$_{419}$	Thr$_{645}$
Arg$_{422}$	Glu$_{464}$
Asp$_{423}$	Arg$_{467}$
Leu$_{424}$	Lys$_{530}$
Phe$_{425}$	Phe$_{482}$
Phe$_{425}$	Lys$_{475}$

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3. A method to identify an agent which modulates the interaction between pRb and $E2F_{(409-426)}$, the method comprising:

a) combining together pRb, $E2F_{(409-426)}$ and an agent, under conditions in which pRb and $E2F_{(409-426)}$ form a complex;

b) growing a crystal structure of any pRb/ $E2F_{(409-426)}$ complex; and

c) analysing the crystal to determine whether the agent is an agent which modulates the interaction between pRb and $E2F_{(409-426)}$.

4. A method, as claimed in claim 3, wherein the combining of the components is pRb with the agent and then $E2F_{(409-426)}$.

5. A method as claimed in claim 3, wherein the combining of the components is $E2F_{(409-426)}$ with the agent and then pRb.

6. A method as claimed in claim 3, wherein the combining of the components is pRb with $E2F_{(409-426)}$ and then the agent.

7. A method as claimed in any one of claims 3 to 6, wherein step c) comprises comparing the crystal structure to the crystal structure of claim 1

8. A method as claimed in any one of claims 3 to 7, wherein the agent is selected using the three dimensional atomic co-ordinates of Annex 1.

9. A method of identifying an agent that modulates a pRb/$E2F_{(409-426)}$ complex, comprising selecting an agent using the three-dimensional atomic coordinates of Annex 1.

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10. A method as claimed in claim 9, wherein said selection is performed in conjunction with computer modeling.

11. A method as claimed in claim 9 or 10, wherein the method further comprises the steps of:

a) contacting the selected agent with pRb and $E2F_{(409-426)}$ under conditions in which pRb and $E2F_{(409-426)}$ can form a complex; and

b) measuring the binding affinity of pRb to $E2F_{(409-426)}$ in the presence of the agent and comparing the binding affinity to that of pRb to $E2F_{(409-426)}$ when in the absence of the agent, wherein an agent modulates a pRb/$E2F_{(409-426)}$ complex when there is a change in the binding affinity of pRb to $E2F_{(409-426)}$ when in the presence of the agent.

12. A method as claimed in claim 11, wherein the method further comprising:

a) growing a supplementary crystal from a solution containing pRb and $E2F_{(409-426)}$ and the selected agent where said agent changes the binding affinity of the pRb/$E2F_{(409-426)}$ complex under conditions in which pRb and $E2F_{(409-426)}$ can form a complex;

b) determining the three-dimensional atomic coordinates of the supplementary crystal by X-ray diffraction using molecular replacement analysis;

c) comparing the three dimensional coordinates with those for the crystal structure as claimed in claim 1; and

d) selecting a second generation agent using the three-dimensional atomic coordinates determined for the supplementary crystal.

13. A method as claimed in claim 12, wherein said selection is performed in conjunction with computer modeling.

14. A method of identifying an agent that modulates a pRb/$E2F_{(409-426)}$ complex, comprising:

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a) contacting a selected agent with pRb and $E2F_{(409-426)}$ under conditions in which pRb and $E2F_{(409-426)}$ can form a complex; and

b) measuring the binding affinity of pRb to $E2F_{(409-426)}$ in the presence of the agent and comparing the binding affinity to that of pRb to $E2F_{(409-426)}$ when in the absence of the agent, wherein an agent modulates a pRb/$E2F_{(409-426)}$ complex when there is a change in the binding affinity of pRb to $E2F_{(409-426)}$ when in the presence of the agent.

15. A method as claimed in claim 14, wherein the method further comprising:

a) growing a supplementary crystal from a solution containing pRb and $E2F_{(409-426)}$ and the selected agent where said agent changes the binding affinity of the pRb/$E2F_{(409-426)}$ complex under conditions in which pRb and $E2F_{(409-426)}$ can form a complex;

b) determining the three-dimensional atomic coordinates of the supplementary crystal by X-ray diffraction using molecular replacement analysis;

c) comparing the three dimensional coordinates with those for the crystal structure claimed in claim 1; and

d) selecting a second generation agent using the three-dimensional atomic coordinates determined for the supplementary crystal.

16. A method as claimed in claim 15, wherein said selection is performed in conjunction with computer modeling.

17. A method of identifying an agent that modulates a pRb/$E2F_{(409-426)}$ complex, comprising:

a) selecting an agent;

b) co-crystalising pRb with the agent;

c) determining the three dimensional coordinates of the pRb-agent association by X-ray diffraction using molecular replacement analysis; and

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d) comparing the three dimensional coordinates with those of the crystal structure claimed in claim 1.

18. A method of identifying an agent that modulates a pRb/E2F$_{(409-426)}$ complex, comprising:

a) selecting an agent;

b) crystalising pRb and soaking the agent into the crystal;

c) determining the three dimensional coordinates of the pRb-agent association by X-ray diffraction using molecular replacement analysis; and

d) comparing the three dimensional coordinates with those of the crystal structure claimed in claim 1.

19. A method of identifying an agent that modulates a pRb/E2F$_{(409-426)}$ complex, comprising:

a) selecting an agent;

b) co-crystalising pRb, E2F$_{(409-426)}$ and the agent;

c) determining the three dimensional coordinates of the pRb-E2F-agent association by X-ray diffraction using molecular replacement analysis; and

d) comparing the three dimensional coordinates with those of the crystal structure claimed in claim 1.

20. A method of identifying an agent that modulates a pRb/E2F$_{(409-426)}$ complex, comprising:

a) selecting an agent;

b) co-crystalising pRb and E2F$_{(409-426)}$ and soaking the agent into the crystal;

c) determining the three dimensional coordinates of the pRb-E2F-agent association by X-ray diffraction using molecular replacement analysis; and

d) comparing the three dimensional coordinates with those of the crystal structure claimed in claim 1.

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21. A method as claimed in any one of claims 17 to 20, wherein the agent is selected using the three dimensional atomic co-ordinates of Annex 1

22. A method as claimed in any one of claims 17 to 21, wherein the methods further comprise selecting a second generation agent using the three dimensional atomic coordinates determined in step c).

23. A method as claimed in claim 22, wherein the second generation agent is selected using the three dimensional atomic coordinates of Annex 1.

24. A method as claimed in claim 22 or 23, wherein the selection is performed in conjunction with computer modeling.

25. A method of identifying an agent as claimed in any one of claims 3 to 24, wherein the selected agent and/or the second generation agent mimics a structural feature of $E2F_{(409-426)}$, when said $E2F_{(409-426)}$ is bound to pRb.

26. A method as claimed in claim 9 or 10, wherein method comprises the further steps of:

a) contacting the selected agent with a pRb/$E2F_{(409-426)}$ complex; and
b) determining whether the agent affects the stability of the complex.

27. A method as claimed in claim 26, wherein the determination is with fluorescence polarization.

28. A method of identifying an agent that modulates a pRb/$E2F_{(409-426)}$ complex, comprising:

a) contacting a fluorescently tagged $E2F_{(409-426)}$ peptide (E2F-fluoropeptide) with pRb to allow pRb/E2F-fluoropeptide complex formation;
b) detecting the fluorescence polarization;

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c) adding a selected agent; and

d) detecting the fluorescence polarization in the presence of the agent.

29. A method of identifying an agent that modulates a pRb/$E2F_{(409-426)}$ complex, comprising;

a) contacting a fluorescently tagged $E2F_{(409-426)}$ peptide (E2F-fluoropeptide) with pRb to allow pRb/E2F-fluoropeptide complex formation;

b) detecting the fluorescence polarization;

c) contacting a selected agent with pRb and $E2F_{(409-426)}$ peptide (E2F-fluoropeptide) under conditions in which pRb and E2F-fluoropeptide can form a complex;

d) detecting the fluorescence polarization; and

e) comparing the fluorescence polarization detected in b) and d).

30. A method as claimed in claim 28 or 29, wherein the fluorescently tagged E2F peptide is selected using the three dimensional atomic co-ordinates of Annex 1.

31. A method as claimed in any one of claims 28 to 30, wherein a decrease in fluorescence polarization in the presence of the agent indicates that the agent destabilises the complex.

32. A method as claimed in any one of claims 28 to 31, wherein the method comprises the further step of adding untagged $E2F_{(409-426)}$ and detecting fluorescence polarization.

33. A method as claimed in claim 32, wherein if fluorescence polarization decreases, on addition of the untagged $E2F_{(409-426)}$, the agent does not stabilise the complex.

34. A method as claimed in claim 32 or 33, wherein if there is no substantial change in fluorescence polarization, on addition of the untagged $E2F_{(409-426)}$, the agent stabilises the complex.

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35. A method as claimed in any one of claims 28 to 34, wherein the method further comprises:

a) contacting a fluorescently tagged E7 peptide (E7-fluoropeptide) with pRb to allow pRb/E7-fluoropeptide complex formation;

b) detecting the fluorescence polarization;

c) adding an agent that modulates pRb/$E2F_{(409-426)}$ complex; and

d) detecting the fluorescence polarization in the presence of the agent.

36. A method as claimed in any one of claims 28 to 34, wherein the method further comprises:

a) contacting a fluorescently tagged E7 peptide (E7-fluoropeptide) with pRb to allow pRb/E7-fluoropeptide complex formation;

b) detecting the fluorescence polarization;

c) contacting an agent that modulates pRb/$E2F_{(409-426)}$ complex with pRb and E7-fluoropeptide under conditions in which pRb and E7-fluoropeptide can from a complex;

d) detecting the fluorescence polarization; and

e) comparing the fluorescence polarization detected in b) and d).

37. A method as claimed in claim 35 or 36, wherein a decrease in fluorescence polarization indicates that the agent also inhibits E7 binding to pRb.

38. A method as claimed in any one of claims 11 to 16, wherein the binding affinity is measured by isothermal titration calorimetry.

39. A method as claimed in any one of claims 11 to 16, wherein the binding affinity is measure by Surface Plasmon Resonance (SPR).

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40. An agent, that modulates the interaction between pRb and $E2F_{(409-426)}$, identified by a method as claimed in any one of claims 3 to 39.

41. An agent, as claimed in claim 40, for use as an apoptosis promoting factor in the prevention or treatment of proliferative diseases.

42. An agent as claimed in claim 40 or 41, wherein the agent is for use in preventing or treating cancer, which may be pancreatic cancer and related diseases.

43. The use of an agent, which modulates the formation of a $pRb/E2F_{(409-426)}$ complex, identified by a method as claimed in any one of claims 3 to 39, in the manufacture of a medicament for the prevention or treatment of proliferative diseases.

44. The use of an agent as claimed in claim 43, wherein the proliferative diseases are cancer, preferably pancreatic cancer and related diseases.

45. The use of the atomic co-ordinates of the crystal structure as claimed in claim 1 or 2, for identifying an agent that modulates the formation of a $pRb/E2F_{(409-426)}$ complex.

46. Computer readable media comprising a data storage material encoded with computer readable data, wherein said computer readable data comprises a set of atomic co-ordinates of the $pRb/E2F_{(409-426)}$ crystal structure of Annex 1 recorded thereon.

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